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Urea and renal concentrating ability in the rabbit

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Urea and renal concentrating ability in the rabbit. The hypotheses of passive salt accumulation predict an enhancement of renal concentrating ability by urea. We tested this prediction in rabbits, a species whose nephrons when studied in vitro show transport properties that support these hypotheses. We used calm, unanesthetized, hydropenic, vasopressin-treated rabbits with intact kidneys fed a 16% protein diet, and we observed the effect of urea administration at two rates of solute excretion (60 and 190 μOsm/min · kg body wt; N = 10 and 5, respectively). After an i.v. mannitol infusion, when urea was infused, the i.v. solute excretion rate was unchanged, the changes in urine urea concentration were large (a change of 767 and 408 μmoles/ml), but only small and variable changes in urine osmolality occurred (a change of 78 ± 146, and 36 ± 50 μOsm/g H_2O). In additional experiments, we removed the kidneys from antidiuretic, or urea- or mannitol-infused rabbits and measured the intrarenal distribution of sodium, potassium, urea, and chloride. When the urine urea level was greater than 400 mmoles, the urine-to-papilla ratios for urea were 1.6 to 3.6. This suggested that a low collecting duct permeability to urea could explain the absence of a marked enhancement of concentrating ability during urea administration. Further analysis, based on a model of inner medullary solute compartments, indicated that sodium chloride was the major (86%) osmotically active solute in the medullary central core of these rabbits and that it was not influenced by changes in urinary urea concentration. The results of tissue analysis were consonant with either active or passive sodium chloride reabsorption from the thin ascending limb of Henle's loop in these rabbits.

Urée et pouvoir de concentration rénal chez le lapin. Les hypothèses d'accumulation passive de sel impliquent une augmentation de la capacité de concentration par l'urée. Cette implication a été étudiée chez le lapin, une espèce dont les néphrons ont des propriétés de transport in vitro qui sont en accord avec ces hypothèses. Des lapins nourris avec 16% de protéines, intacts, clames, non anesthésiés, privés d'eau et traités par la vasopressine ont été étudiés. L'effet de l'administration d'urée a été observé à deux débits d'excretion de substances dissoutes (60 et 190 μOsm/min · kg de poids corporel; N = 10 et 5, respectifment). Après la perfusion de mannitol i.v., la perfusion d'urée n'a pas modifié le débit d'excretion de substances dissoutes, a augmenté considérablement la concentration urinaire de l'urée (modification de 767 et 408 μmoles/ml), mais n'a entraîné que des modifications mineures et variables de l'osmolalité urinaire (modification de 78 ± 146 et 36 ± 50 μOsm/g H_2O). Au cours d'autres expériences des reins ont été prélevés chez des lapins en antidiurèse ou en perfusion d'urée ou de mannitol, et la distribution intrarénale de sodium, potassium, urée et clorure a été mesurée. Quand la concentration urinaire d'urée est supérieure à 400 mmoles le rapport de concentration de l'urée urine sur papille (1,6 à 3,6) suggère qu'une faible perméabilité à l'urée du canal collecteur pourrait expliquer l'augmentation importante de la capacité de concentration au cours de l'administration d'urée. Une analyse plus poussée, fondée sur un modèle de compartiments médullaires internes pour les substances dissoutes, a indiqué que chlorure de sodium était la principale substance dissoute osmotiquement active (86%) dans la partie centrale de la médullaire chez ces lapins et n'était pas influencée par les modifications de l'urée urinaire. Les résultats obtenus sur les fragments tissulaires sont compatibles avec une réabsorption, active ou passive, de chlorure de sodium dans la branche grêle ascendante de l'anse de Henle.

A major, unresolved question concerning the renal concentrating mechanism is whether salt (sodium or chloride) is reabsorbed actively or passively from the thin ascending limb of the loop of Henle (TAL) [1–4]. The concentration of sodium chloride increases along the cortex-to-papilla axis in the inner medulla, and this requires net reabsorption of sodium chloride from the TAL [5]. Evidence for the presence of active sodium chloride reabsorption from the TAL is mixed, and disagreement exists whether it is present at all [1]. Stephenson has shown how active sodium chloride transport in the TAL could be supplemented or replaced by a passive sodium chloride transport system [6, 7]. Kokko and Rector have presented a unique case of the Stephenson model, in which only passive salt reabsorption occurs from the TAL [8]. Evidence for these passive models arises primarily from studies of the transport properties of isolated segments of rabbit renal tubules studied in vitro [9–11]. In vivo evidence from micropuncture studies in other species often has failed to confirm the conclusions drawn from the rabbit studies [1–3, 12–18]. Berliner has suggested that mammalian species may differ, with the rabbit possessing but the hamster or Psammomys not possessing the passive salt reabsorptive mechanism [2].

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This suggestion prompted our study on unanesthetized rabbits with intact kidneys and normal renal function. Our aim was to collect evidence pertaining directly to the presence of the passive salt reabsorptive mechanism proposed by Stephenson [6] and by Kokko and Rector [8]. Both Berliner [2] and Stewart and Valtin [19] had indicated that the passive salt reabsorption model would predict the well-known enhancement of renal concentrating ability by urea that previously has been demonstrated experimentally in dog, rat, and human [20–33]. We reasoned that if the passive salt reabsorption model applied to the rabbit, the enhancement of renal concentrating ability by urea should be demonstrable in this species. To test this proposition, we chose an experimental design that previously had been used successfully to demonstrate acute elevations of urine osmolality referable to urea administration in both dog and rat [30, 31]. In thirsted, vasopressin-treated animals, first, mannitol is infused to produce a given rate of solute excretion with a low concentration of urea in the urine. Then urea is infused to obtain the same total solute excretion rate but also to raise greatly the urinary urea concentration. Previously, in the dog and rat fed normal protein diets, the urea infusion led to acute increases in urine osmolality that paralleled the increases in the urine urea concentration [30, 31]. The proposition underlying the present study is that a failure of urea administration to produce enhancement in the rabbit would indicate the absence of one or more processes critical to the working of the passive salt reabsorptive mechanism as described by Stephenson and by Kokko and Rector.

After we observed that urea administration failed to produce a significant enhancement of concentrating ability in unanesthetized rabbits, we performed additional studies that involved the measurement of the sodium, chloride, potassium, and urea concentrations of renal tissue, urine, and plasma of rabbits in various states of antidiuresis and diuresis. The purpose of these additional experiments was to obtain evidence regarding the nature of some of the processes involved in the accumulation of solute in the inner medullary central core (interstitium and vasa recta).

Methods

Forty-eight experiments were performed on 16 female, adult, nonpregnant New Zealand white rabbits (3.34 ± 0.28 kg body wt), with one to seven experiments performed on an individual rabbit.

For at least 14 days before an experiment, each animal was fed 150 g/day of Purina rabbit chow (0.32% sodium, 1.51% potassium, and 16.2% protein). Water was given ad lib until 24 hours before a study when it was removed. All solutions that were given by constant infusion contained vasopressin (Sigma Chemical Co.) and provided antidiuretic hormone (ADH) at 0.1 mU/min/kg body wt. During each experiment, a rabbit was unanesthetized and securely held in a plastic restraining cage. Urine was continuously collected from the bladder by way of a rubber retention catheter (Mallecott modified at the tip, French size 12) in graduated glass cylinders. The catheter was inserted through the urethra when the animal was in the restraining cage, and the pudendal region was anesthetized by a topical spray (Cetacaine®, Haver-Lockhart Laboratories). Solutions were administered through a catheter inserted in one lateral ear vein. The ear-vein catheter was a size 23-gauge needle, without hub, attached to a length of polyethelene tubing. Constant infusion of solutions was made from a constant infusion syringe pump (Harvard Apparatus Co.). In most experiments, blood samples of 0.7 to 1.0 ml were collected into a heparinized syringe at the midpoint of either all or of occasional urine collection periods from a catheter placed in the central artery of a contralateral ear. The arterial catheter was either a 25- or 23-gauge needle, without hub, attached to polyethylene tubing. The local injection of approximately 0.1 ml of 2% lidocaine hydrochloride made insertion of the vascular catheters nontraumatic and apparently painless. Clotting at the tip of the arterial catheter was prevented during the experiment by infusing at 0.007 ml/min a 0.9% sodium chloride solution containing 7.0 U/ml sodium heparin. In preliminary experiments and in the first series reported below, the rabbits were obviously tense and frightened and occasionally struggled when in the restraining cage. This behavior was subsequently altered to one of calm, acquiescent restfulness after the rabbits were conditioned, prior to study, to the experimenters, the laboratory, and the restraining cage. After being allowed to roam the laboratory at will and after repeated handling and several periods, each lasting 1 to 8 hours, in a restraining cage, the rabbits rarely showed fear or opposition to restraint during an experiment. Urine flow, which was quite variable in preliminary experiments and in some experiments of group 1, became stable in the conditioned animals used in the experiments of groups 2 to 4.

At the end of each experiment, the rabbit received a 0.4-ml i.m. injection of antibiotic (Combiotic®, 200,000 U/ml procaine penicillin G and 250 mg/ml dihydrostreptomycin sulfate; Chas. Pfizer, Co.). The bladder was washed out with a dilute solution of nitrofurazone to control possible bladder infection. All i.v. solutions were sterilized either by autoclaving or by filtration through a 0.45-μm pore filter.

The experiments are divided into four major groups, according to purpose. The number of animals studied and the details of the procedures are given in Table 1.

Group 1. Experiments were performed to evaluate the relation of urine osmolality (U_{Osm}) to the total solute excretion rate ($U_{Osm}V$) during a stepwise increase in solute excretion caused by a stepwise infusion of either mannitol or urea.

Group 2. These experiments tested the effect of urea on renal concentrating ability and involved the infusion of mannitol followed immediately thereafter by infusion of urea. The comparisons between urea and mannitol infusions were made at two levels of total solute excretion, one providing urine osmolality values in the range of 1200 to 1800 mOsm/kg H_2O (*low-solute excretion rate experiments*), and the other at a solute excretion providing urine osmolality in the range of 500 to 800 mOsm/kg H_2O (*high-solute excretion rate experiments*). In the group 2 experiments, the mannitol infusion was prolonged until at least three consecutive collections were obtained in which urine osmolality varied by less than 10%. Urea infusion was prolonged until two criteria were met: There were at least three consecutive collections in which the solute excretion rate varied by less than ± 15%; and the solute excretion rate in at least one of the three periods was within the range of the last three mannitol infusion periods. Urea is cleared appreciably from rabbit plasma by metabolism of enteric organisms

Table 1. Experimental procedures

Experimental group	Solute infused[a]	Duration collection periods *min*	Duration infusion *min*	Infusion rate *ml/min*	Solute concentration *mmoles*
1. Stepwise mannitol diuresis (N = 3)	Mannitol[b]	20	80	0.123[c]	700
	Mannitol[b]	20	80	0.286[d]	600
	Mannitol[b]	10	40	0.558[d]	500
	Mannitol[b]	5	30	1.38	350
Stepwise urea diuresis[e] (N = 2)					
2. Mannitol-to-urea experiments					
Low-solute excretion rates (N = 10)					
	Mannitol[f]	20	120 to 140	0.14[g]	650
	Urea[f]	20	180	0.28[h]	900
High-solute excretion rates (N = 5)					
	Mannitol[f]	5 to 10	60 to 90	1.09	350
	Urea[f]	5 to 10	70 to 130	1.4	500 or 650
3. Control experiments					
Mannitol-to-mannitol (N = 4)	Mannitol[f]	20	120 to 160	0.14[g]	650
	Mannitol[f]	20	180	0.28[h]	350
Mannitol-to-Na_2SO_4 (N = 3)	Mannitol[f]	5 to 10	60 to 90	1.09	350
	Na_2SO_4[f]	10	80 to 120	1.05	163
Mannitol-to-PAH (N = 4)	Mannitol[f]	20	120 to 160	0.14[g]	650
	PAH	20	140 to 180	0.14	510
4. Tissue studies					
Antidiuresis (N = 4)	NaCl	30 to 40	170	0.01	155
Low-urea infusion (N = 5)	Urea[f]	20	200 to 240	0.15[i]	900
High-urea infusion (N = 2)	Urea[f]	5 to 10	115 to 120	1.4	900
Low-mannitol infusion (N = 3)	Mannitol[f]	20	180	0.15[i]	650
High-mannitol infusion (N = 3)	Mannitol[f]	10 to 20	160 to 185	1.0	350

[a] Priming infusions contained no vasopressin but were otherwise identical to the associated infusion solution.
[b] With 75 mmoles of sodium chloride
[c] Priming infusion of 1.8 ml
[d] Priming infusion of 2.8 ml
[e] Procedure was similar to stepwise mannitol experiments except that urea was substituted for mannitol.
[f] With 100 mmoles of sodium chloride
[g] Priming infusion of 2.1 ml
[h] Priming infusion of 4.2 ml
[i] Priming infusion of 2.0 ml

[35]. Obtaining comparable rates of solute excretion during mannitol and urea infusions required infusion of greater amounts of urea than mannitol. Numerous preliminary experiments were performed to arrive at the selected infusion protocols listed in Table 1.

Group 3 (control experiments): (A) Mannitol infusion followed by either sodium sulfate, PAH, or mannitol infusion. The design of the *group 2* experiments relies on the assumption that mannitol is representative of nonurea solutes in general in its effect on concentrating ability in the rabbit. This assumption was tested by replacing urea by sodium sulfate (high-solute infusion protocol) or by replacing urea by PAH (low-solute infusion protocol).

The design of the *group 2* experiments also relies on an assumption that the duration of the experiment and the alterations in flow and osmolality that occurred during the transition from the mannitol to urea infusions did not significantly alter concentrating ability. This assumption was tested by interrupting the mannitol infusion, giving a second mannitol priming infusion, and reinstating mannitol infusion at a higher flow rate (low-solute infusion protocol).

(B) Measurements of GRF and renal plasma flow (RPF). In four mannitol-to-urea low-infusion experiments of *group 2* and in the four mannitol-to-mannitol experiments of *group 3*, the GFR and RPF were estimated by measuring the clearances of inulin and PAH. In these experiments, a solution containing inulin (4 g/dl), PAH (5 g/dl), and sodium chloride (0.9 g/dl) was initially given as a 3.0-ml priming injection and then as a constant infusion at 0.07 ml/min.

Group 4. The *fourth* group of experiments were done to measure the intrarenal distribution and the urine-to-papilla concentration differences of urea and other substances in rabbits. An attempt was made to obtain wide variations in flow rates, in urine urea, and in urine osmolar concentrations. Hence, kidneys were obtained from thirsted, antidiuretic-hormone-treated rabbits that were antidiuretic or infused at low or at high rates with mannitol or urea (Table 1). All of the experiments were allowed to proceed until urine osmolality stabilized with changes less than 5% for three consecutive periods. The terminal arterial blood sample was taken during the last 2 min of the terminal urine collection period.

At the end of these clearance-type experiments, each rabbit was rapidly anesthetized by i.v. injection of sodium pentobarbital, and one kidney was rapidly removed through a flank incision and quickly frozen in a dry-ice and acetone mixture. The kidney tissue was kept frozen while each pole was cut off and while the remaining central section was trimmed to leave a strip approximately 4- to 6-mm thick that extended from the outer cortical capsule to the tip of the papilla. While still frozen, this central piece was further cut into sections as follows: C-1, outer cortex; C-2, inner cortex; OM-1, outer zone of outer medulla; OM-2, inner zone of outer medulla; IM-1, outer portion of inner medulla; IM-2, middle portion of inner medulla; and IM-3 inner portion of the inner medulla (the projecting renal papilla). While frozen, the tissue sections were weighed. The final tissue section weights were approximately 30 to 80 mg (inner medulla) and 100 to 300 mg (remaining pieces). Each tissue section was then dried under vacuum while frozen, then reweighed; water content was computed. Each dried tissue section was then homogenized in 1.0-ml glass-distilled water in a small glass homogenizer. Immediately after homogenization, 0.2 ml of homogenate was quantitatively removed to a 2.0-ml volumetric tube containing 1 ml of water. Zinc sulfate and sodium hydroxide were added to precepitate proteins, and water was then added to bring the final volume to 2.00 ml. After its centrifugation, the supernatant fluid was separated and subsequently analyzed for urea. Another portion of the homogenate (0.6 ml) was transferred to a 2.0-ml volumetric tube, and 0.5 ml of concentrated nitric acid was added. The mixture was warmed until digestion produced clarity. Then, distilled water was added to volume, and the solution analyzed for sodium and potassium. Chloride was directly measured on the homogenate.

The concentrations of urea, sodium, potassium, and chloride were calculated for each piece of tissue by using the value of tissue water measured on that piece and were expressed in micromoles per gram of tissue water or microequivalents per gram of tissue water. Concentrations in urine and plasma were expressed in micromoles per milliliter or microequivalents per milliliter, and differences in concentration between tissue and urine or plasma were expressed micromoles per milliliter or microequivalents per milliliter. The reported urine and plasma concentrations were from terminal collection periods.

Analyses. Urine and plasma osmolality was measured using a vapor pressure osmometer (Wescor Co.). Methods used for measurement of urea, sodium, potassium, chloride, inulin, and PAH have

been reported [36]. Because Cetacaine produces additional color in the PAH determination, this topical anesthetic was not applied in experiments when PAH clearance was measured. Means ± SD are reported; Student's *t* test was used to test significance. *P* greater than 0.05 is reported as nonsignificant (NS). Linear regression was determined by the method of least-squares.

Results

General observations. Preconditioning of the rabbits led to their calm behavior, and only rare agitation during an experiment. Urine flow during a constant infusion was relatively invariant. Occasional period-to-period variations in flow were attributable to incomplete bladder emptying because simultaneously measured osmolality and urea concentrations remained unchanged. Inulin and PAH clearances were stable when measured in the four mannitol-to-urea and the four mannitol-to-mannitol experiments (Table 2).

During the stepwise infusion of either mannitol or urea, urine osmolality declined as solute excretion rate increased. This is illustrated for one experiment with mannitol infusion in Fig. 1. A similar relationship can be inferred from the observations in rabbits of Barraclough, Guignard, and Jones [37]. This pattern is common to other mammals and indicates the basic similarity of the concentrating mechanism of rabbits and other mammals. It also indicates the necessity of maintaining the urine solute excretion rate constant when attempting to evaluate an effect of urea on concentrating ability. In rabbits studied during a progressive solute infusion on different days, the pattern of response was similar but the absolute values of urine osmolality, at any given rate of solute excretion, varied significantly from day to day and from rabbit to rabbit. Therefore, it was considered necessary to evaluate the effects of urea administration in individual rabbits on a single day, and the protocol used in the group 2 and 3 experiments was adopted.

Figure 1 also indicates that the highest urine osmolality should be observed at the lowest solute excretion rate. In 25 hydropenic rabbits, when bladder urine was collected prior to infusion of any solute or antidiuretic hormone (ADH), there was considerable urinary precipitate present. The mean osmolality of this bladder urine was 1504 ± 327 μOsm/g H_2O (range, 947 to 2084 μOsm). Due to removal of urinary solutes from solution into the precipitate, these values constitute a minimum estimate of the concentrating ability of the hydropenic rabbit maintained on a 16% protein diet. The precipitate disappeared from the urine, with few exceptions, once the rabbits were infused with solute to increase the total solute excretion rate.

Group 2 (mannitol-to-urea) experiments. Results obtained in the terminal period of mannitol infusion (100 to 120 min after beginning mannitol infusion) and in a urea infusion period ending 120 to 140 min after beginning the urea infusion are compared in Table 3 (individual experiments) and Table 4 (mean values for each group of experiments). Infusion of urea after mannitol consistently produced significant increases in the urine urea concentration and in the plasma urea concentration and plasma osmolality. With urea infusion there was typically an initial rapid rise in urine urea concentration that then tended to reach a plateau in the terminal peri-

Table 2. Inulin and PAH clearances in unanesthetized, restrained rabbits during mannitol and urea infusion[a]

Experiment/rabbit	C_{In} *ml/min · kg body wt*		C_{PAH} *ml/min · kg body wt*	
Group 2: Mannitol-to-urea (4 experiments)	*Mannitol*	*Urea*	*Mannitol*	*Urea*
Rabbit 3967	3.48	3.63	14.9	13.4
Rabbit 4086	4.53	—[b]	—[b]	—[b]
Rabbit 3964	4.56	4.50	14.1	13.3
Rabbit 4088	3.87	4.48	15.76	18.6
Mean ± SD	4.11 ± 0.53	4.2 ± 0.5	14.9 ± 0.8	15.08 ± 3.04
P (difference mannitol and urea)	NS		NS	
Group 3: Mannitol-to-mannitol (4 experiments)	*1st mannitol*	*2nd mannitol*	*1st mannitol*	*2nd mannitol*
Rabbit 3967	3.74	4.02	16.8	18.4
Rabbit 4086	3.94	3.55	14.58	14.25
Rabbit 4086	4.18	4.91	15.33	18.26
Rabbit 3964	3.61	3.81	14.29	14.18
Mean ± SD	3.86 ± 0.25	4.07 ± 0.59	15.3 ± 1.1	16.2 ± 2.4
P (difference 1st and 2nd mannitol)	NS		NS	

[a] Values from individual experiments are the mean of three or four consecutive periods.
[b] Blood samples lost

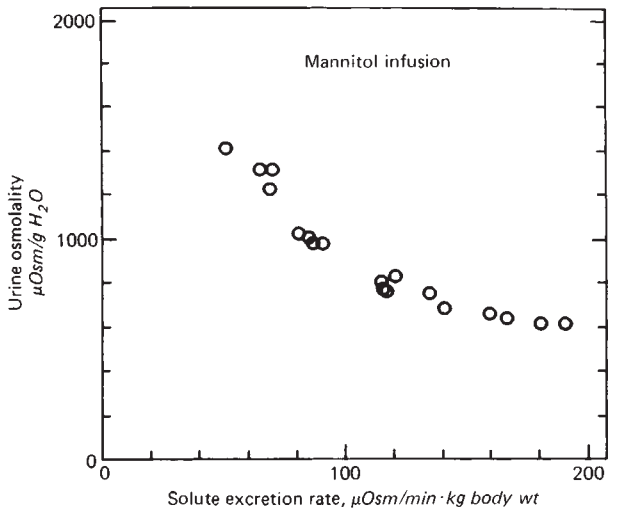

Fig. 1. *Relation between urine osmolality and total solute excretion rate ($U_{Osm}V$) during stepwise infusion of mannitol in one rabbit.* The curvilinear decline is similar to that observed in other mammals under similar experimental conditions.

ods. The changes in plasma osmolality are referable to the changes in plasma urea concentration, which were small in the low-diuresis experiments but large in the high-diuresis experiments. The urea clearance was only approximately 40% of the GFR in these experiments. To obtain equal rates of total solute excretion during the urea and manitol infusions, it was necessary to produce substantially higher plasma concentration of urea than mannitol. This elevated the plasma osmolality during urea infusion.

The solute excretion rate was commonly stable during the terminal periods of mannitol infusion. With urea infusion, the solute excretion declined initially and then rose to approximate the rates during mannitol infusion. The period-to-period comparison (Table 3) shows that the urine osmolality during urea infusion differed from urine osmolality during mannitol infusion by −166 to +273 (low-infusion experiments) and −20 to +90 μOsm/g H_2O (high-infusion experiments), with increases predominating (in eight out of ten low-diuresis experiments and three out of four high-diuresis experiments). In four of the fourteen experiments, the change in urine osmolality varied with the change in solute excretion in a direction and magnitude predictable from the relation shown in Fig. 1. In the high-diuresis experiments, the elevated plasma osmolality may have contributed to the observed increases in urine osmolality, for there was no significant difference between the urine-to-plasma os-

Table 3. Mannitol-to-urea and mannitol-to-mannitol experiments[a, b]

Treatment/ rabbit	P_{Osm} μOsm/g H_2O			P_{Urea} μmoles/ml			U_{Urea} μmoles/ml			U_{Osm} μOsm/g H_2O			$U_{Osm}V$ μOsm/min · kg body wt		
	Mannitol	Urea	Δ	Mannitol	Urea	Δ	Mannitol	Urea	Δ	Mannitol	Urea	Δ	Mannitol	Urea	Δ
Mannitol-to-urea, low infusion.															
3441	300	310	10	9.53	23.8	14.3	265	1003	738	1225	1457	232	65	59.7	−5.3
3433	305	307	2	7.33	17.34	10	249	868	619	1440	1491	51	57	64	7
3442	314	320	6	6.94	20.9	14	296	847	551	1539	1373	−166	60.7	58	−2
3439	302	312	10	7.3	20.2	13	316	1297	981	1648	1743	95	55.8	56.4	1.4
3437	294	305	6	7.5	22.7	15.2	400.7	1302	901	1893	1919	26	59.6	60.5	0.9
3394	298	304	6	7.59	21.95	14.4	333.8	1131	798	1694	1544	−150	62.5	66.0	3.5
3967	290	296	6	4.79	22.7	17.9	237	1076	839	1480	1653	173	57.5	70.2	12.7
4086	—	—	—	—	—	—	283	1066	783	1579	1734	155	59.7	60.9	1.2
3964	305	318	13	6.19	22.1	15.9	281	1022	741	1523	1622	99	62.8	61.3	−1.5
4087	290	306	16	6.36	—	—	241	964	723	1370	1643	273	57.2	62.0	4.8
Mannitol-to-urea, high infusion															
3439	315	350	35	6.72	53.84	46.3	—	—	—	605	670	65	199	208	9
3437	270	290	20	6.37	41.99	35.62	110	561	451	880	860	−20	175	179	4.0
3440	300	336	36	6.04	53	46.96	90.8	611	520	815	825	10	168	199	31
3441	292	332	40	8.57	58.26	49.69	55	356	301	489	579	90	209	214	5.0
3433	320	350	30	7.59	57.4	49.8	69	429	360	623	690	67	171	205	34
	Mannitol	*Mannitol*	Δ	*Mannitol*	*Mannitol*	Δ	*Mannitol*	*Mannitol*	Δ	*Mannitol*	*Mannitol*	Δ	*Mannitol*	*Mannitol*	Δ
Mannitol-to-mannitol															
3967	291	294	3.0	7.39	6.89	−0.50	273	277	4	1589	1652	63	59.3	66.5	7.20
4086	298	301	3.0	7.13	7.13	0	310	296	−14	1591	1600	9	57.6	66.5	8.90
4086	294	295	1	5.83	5.57	−0.26	245	246	1	1512	1561	49	58.9	64.8	5.90
3964	298	294	−4	6.94	5.77	−1.17	250	219	−29	1413	1424	11	66	65	−1

[a] Initial value for mannitol is from a single, terminal mannitol infusion period, 100 to 120 min after beginning mannitol infusion. Value for urea is from a single urea infusion period, 120 to 140 min after beginning urea infusion. In mannitol-to-mannitol experiments, second mannitol value is from period 80 to 100 min after giving second mannitol priming injection.

[b] Δ value is urea value minus the mannitol value.

Table 4. Results for groups 2 and 3[a]

	P_{Osm} *μOsm/g*	P_{Urea} *μmoles/ml*	U_{Urea} *μmoles/ml*	U_{Osm} *μOsm/g*	$U_{Osm}V$ *μOsm/min · kg body wt*
Mannitol-to-urea					
Low infusion					
Mannitol	300 ± 8[f]	7 ± 1[f]	290 ± 50[g]	1539 ± 183[g]	60 ± 3[g]
Urea	309 ± 7[f]	21 ± 2[e]	1057 ± 154[g]	1617 ± 159[g]	62 ± 4[g]
Δ	8 ± 4[f, k]	14 ± 2[e, h]	767 ± 125[g, h]	78 ± 146[g, k]	2 ± 5[g, k]
High infusion					
Mannitol	299 ± 19[d]	7 ± 1[d]	81 ± 24[c]	682 ± 160[d]	184 ± 18[d]
Urea	331 ± 24[d]	52 ± 6[d]	489 ± 117[c]	725 ± 115[d]	201 ± 13[d]
Δ	32 ± 7[d, h]	46 ± 6[d, h]	408 ± 47[c, i]	36 ± 50[d, k]	17 ± 15[d, k]
Mannitol-to-mannitol					
Mannitol 1	295 ± 3[c]	7 ± 1[c]	269 ± 30[c]	1526 ± 84[c]	61 ± 4[c]
Mannitol 2	296 ± 3[c]	6 ± 1[c]	259 ± 34[c]	1559 ± 98[c]	66 ± 1[c]
Δ	1 ± 3[c, k]	−0 ± 0[c, k]	−10 ± 15[c, k]	33 ± 27[c, k]	5 ± 4[c, k]
Mannitol-to-PAH					
Mannitol	292 ± 8[c]	8 ± 0[c]	339 ± 55[c]	1678 ± 314[c]	60 ± 4[c]
PAH	288 ± 6[c]	8 ± 0[c]	385 ± 58[c]	1830 ± 303[c]	53 ± 7[c]
Δ	−4 ± 6[c, k]	−0 ± 0[c, k]	45 ± 24[c, i]	151 ± 109[c, j]	−7 ± 10[c, k]
Mannitol-to-Na_2SO_4					
Mannitol	313 ± 28[b]	7 ± 0[b]	—	766 ± 145[b]	168 ± 31[b]
Na_2SO_4	314 ± 28[b]	6 ± 0[b]	105 ± 36[b]	863 ± 215[b]	140 ± 18[b]
Δ	1 ± 0[b, j]	0 ± 0[b, k]	—	97 ± 71[b, k]	−27 ± 36[b, k]

[a] Values are the means ± SD. Mean values are the single-period values presented in Table 1 and the corresponding values from individual mannitol-to-PAH and mannitol-to-Na_2SO_4 experiments. Δ denotes difference between first and second infusions (second infusion minus first infusion). *N* denotes number of experiments.

[b] $N = 3$; [c] $N = 4$; [d] $N = 5$; [e] $N = 8$; [f] $N = 9$; [g] $N = 10$.

[h] $P < 0.001$; [i] $P < 0.01$; [j] $P < 0.05$; [k] P = NS.

Table 5. Results of one mannitol-to-urea experiment (low-infusion rate)

Time *min*	Period	V *ml/min · kg*	U_{Osm} *μOsm/g*	$U_{Osm}V$ *μOsm/min · kg*	U_{Urea} *μmoles/ml*	P_{Osm} *μOsm/g*	P_{Urea} *μmoles/ml*
0	*Give mannitol priming injection; begin constant infusion of mannitol, NaCl, and vasopressin*						
0 to 20	1	0.100	928	92.8	244	—	—
20 to 40	2	0.048	1294	63.4	260	—	—
40 to 60	3	0.040	1520	60.7	309	—	—
60 to 80	4	0.036	1693	60.1	342	—	—
80 to 100	5	0.038	1645	63.2	323	298	7.59
100 to 120	6	0.037	1694	62.5	333	—	—
120	*Terminate mannitol infusion, give urea priming injection, begin constant infusion of urea, NaCl, and vasopressin*						
120 to 140	7	0.053	1431	75.7	363	—	—
140 to 160	8	0.033	1757	58.2	686	—	—
160 to 180	9	0.032	1798	57.9	953	—	—
180 to 200	10	0.033	1759	58.4	1099	307	20.5
220 to 220	11	0.034	1705	57.9	1123	—	—
220 to 240	12	0.041	1623	67.2	1139	304	22.0
240 to 260	13	0.043	1544	66.2	1131	—	—

[a] Results for rabbit 3394 (3.38 kg body wt) are used here. Preinfusion bladder urine osmolality was 2001 μOsm/g H_2O.

molality ratio during mannitol and urea infusions in any high-diuresis experiments. This is illustrated in the right panel of Fig. 2 for an experiment in which there was a definite difference in urine osmolality between mannitol and urea infusion periods (left panel). It is particularly relevant to note that although some of the increases in urine osmolality may have been induced by the administration of urea, there was essentially no correlation between the changes in urine urea concentration and the changes in urine osmolality during urea infusion. This is seen in Table 5 for one low-diuresis experiment and in Fig. 3 for two low-diuresis and two high-diuresis experiments.

In nine of the ten low-diuresis experiments, there was a decline in urine osmolality during the last several periods of urea infusion similar to that shown in Table 5. Consequently, a comparison of urine osmolality between mannitol and urea infusion periods based on the mean of the terminal three or four periods of infusion yields a larger difference than the comparison given in Table 4. Comparison on

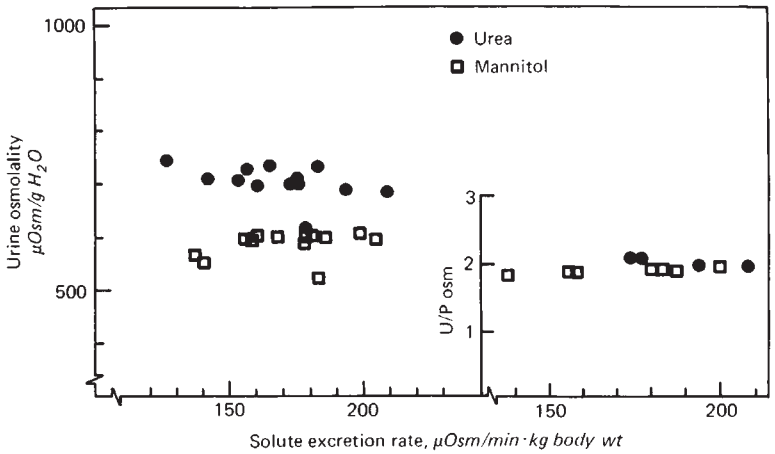

Fig. 2. *Relation of urine osmolality* (left panel) *and urine-to-plasma osmolality ratios* (right panel) *to total solute excretion rate.* Results are from one high-solute infusion experiment in which urea was given after mannitol. Urine-to-plasma osmolality ratios are similar when urea or mannitol is infused; the elevation of urine osmolality during urea infusion, compared to mannitol infusion, is attributed to concomitant increases in plasma osmolality.

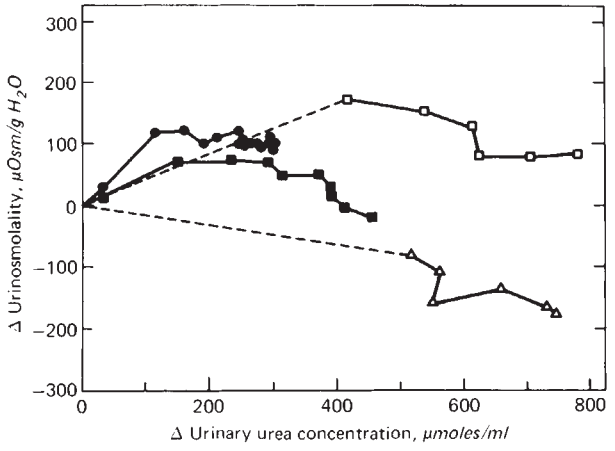

Fig. 3. *Relation of change in urine osmolality during urea administration to change in urinary urea concentration, two low-infusion and two high-infusion experiments.* All points except origin represent difference between mean value during terminal three periods of mannitol infusion and individual periods during urea administration. *Solid lines* connect consecutive periods. Urea was not measured in several initial periods during urea infusion in the two low-infusion experiments (*broken lines*). Low-infusion experiments are denoted by *open symbols*; high infusion experiments, *solid symbols*. Despite large changes in urinary urea concentration, only irregular or small changes in urine osmolality occurred.

this basis gives increases of 130 ± 129 ($N = 10$, $P < 0.02$) and 12.7 ± 22 ($N = 5$, $P = NS$) μOsm/kg H_2O for the low- and high-diuresis groups, respectively.

Group 3 (mannitol-to-mannitol, mannitol-to-PAH, and mannitol-to-sodium sulfate) experiments. Table 3 presents individual results, and Table 4 presents summary results for the mannitol-to-mannitol experiments. In the mannitol-to-mannitol experiments, there were small increases in urine osmolality (9 to 63 μOsm/g H_2O) associated with either unchanged or slightly elevated solute excretion rates (−1 to 8.9 μOsm/min. kg body wt). Otherwise, there was no effect produced by interrupting and changing the infusion and by giving a second priming injection. In the mannitol-to-PAH experiments (low-diuresis) and the mannitol-to-sodium sulfate experiments (high-diuresis), the only significant changes in urine osmolality were those referable to decreases in the solute excretion rate (Table 3).

Group 4 (tissue distribution) experiments. Table 6 presents all individual outer cortex (C-1) and papillary (IM-3) tissue concentrations, the terminal collection period urine flows, solute excretion rates, and urine and plasma concentrations, as well as the respective means for each treatment group. Figure 4 plots the mean plasma, tissue, and urine concentrations for each treatment group. The significant features of this data are: (*1*) For both sodium and urea, an ascending concentration gradient from the corticomedullary junction to the tip of inner medulla was observed in all rabbits in the antidiuretic, low-urea infusion and low-mannitol infusion groups (Fig. 4). When a large osmotic diuresis was produced by urea or mannitol infusion, the gradients were diminished and tended to plateau or drop in the papilla tip. (*2*) The urinary concentration of urea was always greater than the inner medullary (IM-3) urea concentration. The absolute difference in urea concentration (U_{urea} − IM-3_{urea}) varied from 27 to 988 μOsm/ml, was lowest when the urine urea was low, and was highest when the urine urea was high.

The ratio ($U_{urea}/IM\text{-}3_{urea}$) varied between 1.26 and 3.63 and was greatest in the rabbits undergoing the low urea diuresis. (*3*) The urinary concentration of sodium was less than the IM-3 concentration of sodium in all animals. The differences ($U_{Na} - IM\text{-}3_{Na}$) varied between −16 and −410 μEq/ml, and the ratio $U_{Na}/IM\text{-}3_{Na}$ varied between 0.13 and 0.96. (*4*) The intrarenal distribution of chloride, measured only in three antidiuretic rabbits and the two rabbits given a high-urea infusion, was similar to the sodium distribution in those rabbits. (*5*) The urine potassium concentration varied from 10 to 201 μEq/ml. There was essentially no difference between treatment groups in the tissue concentration of potassium. (*6*) Urine osmolality decreased as solute excretion increased, but the urinary urea concentration did not appear to influence urine osmolality appreciably at any rate of solute excretion (Fig. 5). Therefore, the general behavior of the renal concentrating mechanism of the group 4 rabbits was similar to that of the rabbits of groups 1 to 3.

There are a number of reports of the distribution of urea and of sodium in the rabbit kidney [38–46]. These studies differ from the present one in that quick freezing of the kidney was not used before cutting the kidney and that the reported results either included a very small number of rabbits [44, 45], failed to include either both urea and sodium results [42], or failed to include data for urine and plasma concentrations of solutes [38, 40–43, 46]. However, to the extent that these data can be compared with the present results, they appear to closely approximate our findings.

Discussion

Erratic renal function in rabbits during clearance studies has been commonly observed [47]. In this study, renal function in unanesthetized, restrained rabbits resembled normal renal function in other mammals. There was stability of urine flow, constancy of glomerular filtration and renal plasma flow, an ability to produce a highly concentrated urine, and an inverse relation between urine osmolality and total solute excretion rate during hydropenia and ADH administration. We attribute the stability of function to the preconditioning of the rabbits.

A variety of studies have established clearly that kidneys of man, dog, and rat respond to increased urine and plasma urea concentrations with an elevation of urine osmolality that is independent of changes in solute excretion rate or plasma osmolality [20–33]. In man, the enhancement of concentrating ability is limited to low rates of solute excretion [33]; in dog and rat, it is clearly observed from low to high rates of solute excretion, although the absolute increment in osmolality decreases as the osmotic diuresis increases [27, 28].

To evaluate the acute effects of increases in plasma and urine urea concentration on concentrating ability in rabbits, we infused mannitol and then urea. Urine osmolality was compared at similar solute excretion rates. Urine and plasma urea concentrations were low during mannitol infusion and high during urea infusion. This experimental design had been used before to demonstrate an enhancement of renal concentrating ability in the dog and rat by urea and other compounds [27, 30, 31]. The design rests on three major assumptions.

Two of the assumptions are that mannitol is representative of a large number of solutes in terms of its effects on renal concentrating ability and that interruption of the initial mannitol infusion and administration of a second priming injection and a second infusion would not alter concentrating ability. The results of the mannitol-to-mannitol, mannitol-to-PAH, and mannitol-to-sodium sulfate experiments supported these assumptions because the only changes in urine osmolality observed with the second infusion were attributable to changes in either plasma osmolality or solute excretion rate.

The third assumption was that, in the mannitol-to-urea experiments, the chosen rates of solute excretion, the time allowed for urea infusion, and the induced increases in urine and plasma urea concentration would be adequate to permit an effect of urea on concentrating ability to become evident. Two rates of solute excretion were chosen for comparison in the group 2 urea-to-mannitol experiments: one, a low rate, was associated with an initial high urine osmolality during mannitol infusion; the second, a higher rate, was associated with a lower osmolality. The time allowed for an effect of urea to become manifest was as great or greater than the time allowed in our experiments in dog and rat in which marked enhancement occurred [30, 31]. The increases in urine urea and in plasma urea concentration were as great as those in our experiments in dog and rat. Thus, though we cannot exclude the possibility that more prolonged periods of urea infusion or other rates of solute excretion might disclose a different effect of urea on concentrating ability in the rabbit, there is no prior basis for expecting this to occur.

There was no evidence for enhancement of concentrating ability by urea in the group 4 experiments

Table 6. Plasma, urine, and tissue concentrations from group 4 experiments

Treatment/rabbit	Body wt *kg*	V *ml/min·kg*	U_{Osm} *μOsm/g*	P_{Osm} *H_2O*	Urea P *μmoles/ml*	Urea C-1 *μmoles/g H_2O*	Urea IM-3 *μmoles/g H_2O*	Urea U *μmoles/ml*	Sodium P *μEq/ml*	Sodium C-1 *μEq/g H_2O*
Antidiuresis (N = 3)										
Rabbit 3969	3.24	0.0085	2200	301	8.46	12.1	533	1169	140	72
Rabbit 3971	3.36	0.0182	2428	293	8.49	13.4	373	1094	140	82
Rabbit 3973	3.36	0.0093	2303	296	6.36	11.3	585	1115	140	72
Mean	3.32	0.012	2310	297	7.70	12.3	497	1119	140	75
±SD	±0.07	±0.005	±114	±4.0	±1.22	±1.1	±110	43	±0	±6
Low-urea diuresis (N = 5)										
Rabbit 3437	3.63	—	—	290	—	21.5	—	—	144	84
Rabbit 3433	3.24	0.0154	1902	295	15.4	20.7	540	1528	141	79
Rabbit 3442	3.58	0.0447	1349	302	21	26	292	1060	140	81
Rabbit 3439	3.50	0.0500	1364	305	21	30	417	1122	138	73
Rabbit 3440	3.40	0.0448	1489	315	23.4	28.3	428	1118	142	74
Mean	3.47	0.0354	1658	301	20.9[b]	25.3	422	1207	140	78
±SD	±0.16	±0.0155	±371	10	±3.9	±4.1	±88	±215	±2	±5
High-urea diuresis (N = 2)										
Rabbit 3968	3.36	0.310	653	368	76.8	86.7	233	430	140	77
Rabbit 3970	3.54	0.342	713	344	70	79.6	301	473	135	76
Mean	3.45	0.33	683	356	73	83	267	451	138	77
±SD	±0.13	±0.02	±42	±17	±5	±5	±48	±30	±4	±1
Low-mannitol diuresis (N = 3)										
Rabbit 3394	3.18	0.0332	1657	281	4.76	3.60	179	282	140	110
Rabbit 3587	3.54	0.0218	1917	291	7.26	8.30	317	437	140	80
Rabbit 3441	3.54	0.0282	1678	275	5.52	7.23	224	284	140	77
Mean	3.42	0.028	1750	282	5.84	6.37	240	334	140	89
±SD	±0.20	±0.006	±144	8	±1.28	±2.46	±70	±80	±0	±18
High-mannitol diuresis (N = 3)										
Rabbit 3580	3.51	0.248	720	294	5.41	6.33	43.5	70	131	70
Rabbit 3581	3.48	0.261	680	309	4.89	6.21	20.4	53	136	67
Rabbit 3582	3.72	0.174	788	304	5.10	5.70	49.6	79	131	66
Mean	3.57	0.227	729	302	5.13	6.08	37.8	67	132	67
±SD	±0.13	±0.046	±54	±8	±0.26	±0.33	±15.4	±12	±3	±2

[a] C-1 is outer cortex; IM-3 is inner portion of inner medulla (the projecting renal papilla).
[b] N = 4 rabbits.
[c] These abnormally high plasma potassium values are believed to result from an analytical error. Insufficient sample remained for reevaluation.

(Fig. 5), or in the high-solute excretion experiments of group 2 (mannitol-to-urea experiments). In these latter experiments, all changes in urine osmolality could be attributed to changes in plasma osmolality or to changes in solute excretion rate. In the low-solute excretion experiments, there were increases in urine osmolality, which appear to be specifically related to urea administration. These increases could not be explained by changes in solute excretion rate or plasma osmolality. In six out of ten experiments, the increase in urine osmolality exceeded 70 μOsm/g, an increase larger than any that occurred in the control mannitol-to-mannitol experiments. These changes constitute a modest enhancement of renal concentrating ability referable to urea administration.

We doubt whether this enhancement was mediated by the same mechanisms responsible for the enhancement of concentrating ability by urea in dog and rat because of the following differences in response. *(1)* The changes in urine osmolality were not uniform in these rabbits, with a decrease in urine osmolality occurring in some experiments, whereas in the dog and rat elevations in urine osmolality always occurred with urea administration. *(2)* In these rabbits the observed increases in urine osmolality were small (a maximum increase of 273 μOsm/g) and were substantially less than the increases observed in the dog (541 ± 267) and in the rat (576 ± 150). *(3)* In the rabbit, during urea administration, there was frequently an initial rise in urine osmolality followed by a decline of 30 to 100 μOsm/g, whereas in the dog and rat a continuous rise towards some maximum value was typical. *(4)* In the rabbit, during urea administration, there was no clear correlation between the continuously rising urine urea concentration and the urine osmolality, whereas in both the dog and rat the urine osmolality

		Potassium				Chloride			
IM-3 $\mu Eq/g\ H_2O$	U $\mu Eq/ml$	P $\mu Eq/ml$	C-1 $\mu Eq/g\ H_2O$	IM-3 $\mu Eq/g\ H_2O$	U $\mu Eq/ml$	P $\mu Eq/ml$	C-1 $\mu Eq/g\ H_2O$	IM-3 $\mu Eq/g\ H_2O$	U $\mu Eq/ml$
480	391	3.2	98	109	146	114	96	469	349
477	461	3.3	82	86	201	113	86	452	471
498	382	3.4	95	81	176	115	102	499	473
485	411	3.3	92	92	174	114	94	473	431
±11	±43	±0.1	±9	±15	±27	±1	±8	±23	±70
498	265	3.32	87	92	104	—	—	—	—
421	175	—	69	78	93	—	—	—	—
335	132	3.15	83	70	53	—	—	—	—
380	110	4.28	81	53	43	—	—	—	—
423	180	4.55	93	73	60	—	—	—	—
411	172	3.82[b]	85	73	71	—	—	—	—
±60	±59	±0.69	±9	±14	±26	—	—	—	—
188	83	3.45	77	46	10	115	125	288	93
191	100	3.02	79	46	10	113	123	247	108
190	92	3.3	78	46	10	114	124	268	100
±2	±12	±0.2	±2	±0	±0	±2	±2	±29	±11
456	172	3.35	92	111	98	—	—	—	—
473	63	5.70	81	69	111	—	—	—	—
414	167	5.70	85	72	65	—	—	—	—
447	134	4.92	86	84	91	—	—	—	—
±30	±61	±1.35	±5	±23	±23	—	—	—	—
218	120	9.35[c]	83	67	13	—	—	—	—
159	113	8.25[c]	81	41	11	—	—	—	—
172	107	9.05[c]	52	35	19	—	—	—	—
183	113	9.05[c]	72	43	15	—	—	—	—
±31	±7	±0.30	±17	±8	±4	—	—	—	—

regularly rose in parallel with the urine urea concentration. This major difference in the relation of urine osmolality to the urine urea concentration is shown in Fig. 6, which compares the net changes in urine osmolality and in urine urea concentration between terminal mannitol infusion periods and terminal urea infusion periods in the present experiments with rabbits and in our previous experiments with rats.

After noting these differences, it is important to point out basic similarities in the conditions imposed on rabbits, rats, and dogs: all were fed a diet with a high content of protein, all were studied with the same experimental procedure and by the same investigator, all were made hydropenic and given ADH to protect against changes in urine osmolality due to variable, submaximal ADH stimulation. If the mechanisms responsible for enhancement were the same in rabbit as they were in rat and dog, then their effect was greatly diminished and apparently modified by unknown intercurrent factors.

The rabbit is not the only mammal in which urea administration produced little or no increase in urine osmolality. Table 7 shows that the rabbits we tested closely resemble the sheep and differ from rat and dog.

Enhancement of concentrating ability has been demonstrated both in animals fed diets with high contents of protein [27, 28, 32] and in animals fed low or minimal protein diets. In low-protein fed subjects, urea administration partially or completely reversed the diet-induced depression of concentrating ability [20–26, 29]. We wished to examine the effect of urea on the concentrating ability of rabbits fed low protein diets, and we attempted to produce a typical low-protein condition by feeding several different diets with low or no protein content. With these diets, body weight was maintained or only slightly reduced, but plasma and urine urea concentrations were not appreciably lowered nor were GFR, concentrating ability, or fractional urea excretion appreciably reduced (unpublished observations). Aside from suggesting that nocturnal coprophagia probably supplemented dietary nitrogen intake, we cannot explain the failure to induce a characteristic low-protein state.

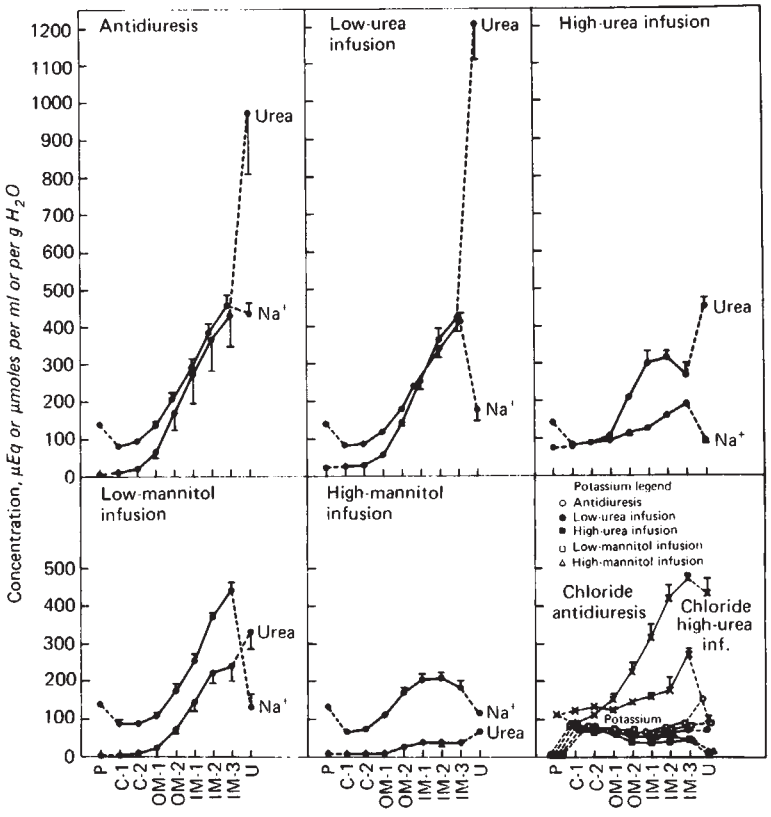

Fig. 4. *Mean plasma, tissue, and urine concentrations.* SEM is shown by vertical bars.

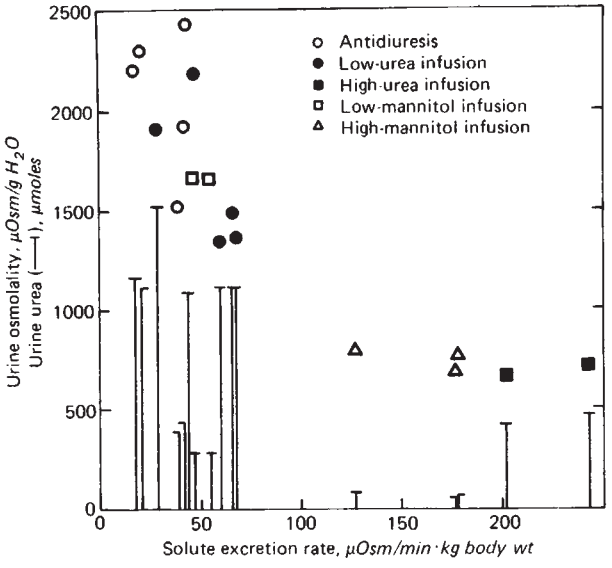

Fig. 5. *Relation of urine osmolality* (symbols) *and urine urea concentration* (vertical lines) *to total solute excretion rate* ($U_{Osm}V$). Presence of urea in high concentration in the urine had no discernible effect on on urine osmolality over a wide range of solute excretion. Values are from terminal collection period in each *group 4* experiment.

The proposition underlying the present study was that enhancement would be demonstrated if all major processes postulated in the passive salt reabsorption hypotheses of Stephenson and of Kokko and Rector were acting in the intact rabbit. The absence of a clearly defined elevation of urine osmolality in parallel with large elevations in the urine area concentration is not in accord with this proposition.

The several processes critical to the working of the passive salt reabsorptive mechanism have been repeatedly listed [1-4, 6-9], and attention here will only be called to the following: *(1)* The urea permeability of the collecting duct is low in the outer medulla and higher in the inner medulla, thus providing for concentration of urea in collecting duct tubular fluid in the outer medulla and substantial passive efflux of urea from collecting duct to central core in the inner medulla. Here, urea will accumulate in a high concentration which approaches the urea concentration in adjacent collecting duct fluid. *(2)* Recycling of urea from collecting duct to interstitium to loop of Henle occurs. *(3)* In the inner me-

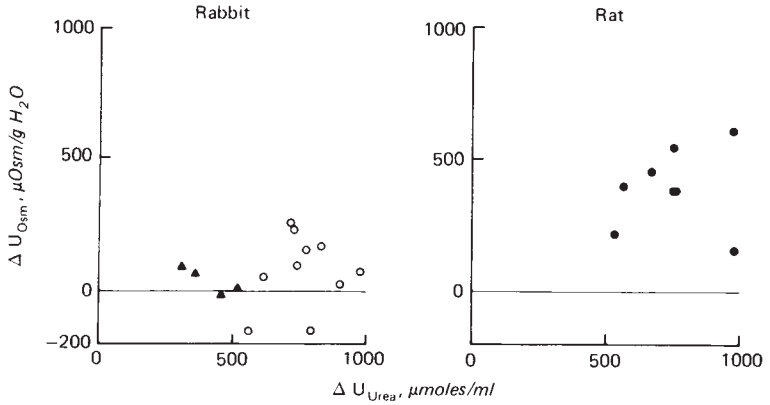

Fig. 6. *Comparison between effect of urea administration in rabbit and rat* [30]. Each point indicates change in urine osmolality (Δ U_{Osm}) and urinary urea concentration (Δ U_{Urea}) between terminal mannitol infusion period and terminal urea infusion period. *Open circles* denote (○) high infusion rabbit studies; *closed triangles*, (▲) low infusion rabbit studies.

dulla, the sodium chloride concentration will be higher within the tubular fluid at the tip of the loop of Henle than it will be in the surrounding central core. This condition arises from the abstraction of water rather than from the addition of solute to fluid in the descending limb of Henle's loop. It provides for the passive efflux of sodium chloride from the thin ascending limb. *(4)* The vasa recta countercurrent exchange system provides for sodium chloride and urea retention in the inner medullary central core. *(5)* Abstraction of water from the thin descending limb requires a high concentration of urea in the inner medullary central core. An important generalization arising from the above processes is that the accumulation of sodium chloride in the inner medullary central core requires the accumulation of urea in high concentration in the central core secondary to the passive efflux of urea from the inner medullary collecting duct.

During the course of the present study, reports have appeared that indicate that the passive salt reabsorption model may not apply to the rabbit. Foster and Jacquez, on the basis of computer modelling of the passive salt reabsorption process, have found that the transport parameters measured in vitro in the perfused rabbit nephron do not provide the anticipated central core salt accumulation but that active salt reabsorption in the rabbit TAL would lead to central core salt accumulation [48]. Stoner and Roch-Ramel have reported [49] results of in vitro perfusion studies of rabbit thin descending limbs that indicate that urea entry may occur in vivo to a greater extent than originally suggested by Kokko [9]. Roch-Ramel et al have found in micropuncture studies in rabbits that recycling of urea from collecting duct to loop of Henle does not occur in superficial nephrons; furthermore, they also concluded from observations of high urine-to-papilla urea ratios in hydropenic rabbits that the inner medullary collecting duct has a low passive permeability to urea and does not allow the attainment of an equilibrium distribution of urea between collecting

Table 7. Comparison of effect of urea administration after mannitol in rabbit, sheep, rat, and dog, all fed high-protein diets[a]

Species	Mannitol administration		Urea administration		Reference
	$U_{Osm}V$[b] *μOsm/min · kg body wt*	U_{Osm} *μOsm/g H_2O*	Δ $U_{Osm}V$ *μOsm/min · kg body wt*	U_{Osm} *μOsm/g H_2O*	
Rabbit					
N = 10	60 ± 3	1539 ± 183	2 ± 5	78 ± 146	Present study
N = 5	184 ± 18	682 ± 160	17 ± 15	36 ± 50	Present study
Sheep (N = 6)	109 ± 9	781 ± 108	−4 ± 18	113 ± 94	[34]
Dog (N = 8)	66 ± 15	713 ± 113	−10 ± 10	526 ± 262	[31]
Rat (N = 9)	190 ± 31	876 ± 97	19 ± 23	500 ± 151	[30]

[a] Values are means ± SD. N is number of animals. Δ is change (urea infusion − mannitol infusion).

[b] Mean solute excretion rates expressed on a metabolic basis (that is, μOsm/min · (kg body wt)$^{3/4}$) are, respectively: rabbit, 80.8 and 248.8; sheep, 289.8; dog, 136.0; and rat, 135.7.

duct and inner medullary central core [50]. Other reports of tissue analyses in rabbits [38–46] had previously indicated a large difference in urea concentration between urine and papilla. Schmidt-Nielsen, O'Dell, and Osaki have suggested that in certain species (pig, beaver, and Psammomys) the permeability of the collecting duct to urea varies inversely with the rate of urea excretion, the collecting duct being relatively impermeable to urea during urea loading [45]. It is not clear whether Schmidt-Nielsen et al wished to include the rabbit among this group of animals; their data from rabbits appears similar to that from Psammomys [45].

The present observations of high urine-to-papilla urea ratios support the inference that urea does not approach equilibrium distribution across the inner medullary collecting duct in the unanesthetized, hydropenic, vasopressin-treated rabbit. We have developed a two-compartment model of the papilla that we use to generate additional quantitative information regarding both the urea distribution across the collecting duct epithelium and the relation of sodium chloride accumulation to urea accumulation in the inner medulla. This model and its quantitative solution is described in the Appendix to this article. The computed concentrations of urea and sodium in central core and of sodium chloride in the loop of Henle are given in Table 8.

The following generalizations arising from the group 4 experiments are based largely on the results in Tables 6 and 8 and the graphic presentation of the measured and computed tissue and urine values of the group 4 experiments (Figs. 7 and 8). The IM-3 urea concentration was less than but approximately proportional to the urine urea concentration, $IM\text{-}3_{urea} = 0.31\ U_{urea} + 90$ ($r = 0.89$). This condition would be expected if most of the urea in the papillary tissue was contained within the collecting duct lumen. The computed mean papillary central core urea concentration was less than either the IM-3 urea concentration or the urinary urea concentration (Fig. 7, A and B), mean central core urea = $0.62\ IM\text{-}3_{urea} + 18$ ($r = 0.92$) and mean central core urea = $0.16\ U_{urea} + 93$ ($r = 0.66$). At low concentrations of urinary urea, the central core urea concentration approaches the urine urea concentration, whereas at urinary urea levels above 350 mmoles, there is a progressive divergence (Fig. 7B). This is more evident in Fig. 7C where the ratio central core urea : urine urea is plotted against the urine urea concentration, mean central core $urea/U_{urea} = -0.0002\ U_{urea} + 0.56$ ($r = 0.67$). The finding of a low ratio at urine urea concentrations above 350 mmoles agrees with the results of Roch-Ramel et al [50] and supports their conclusion that the collecting duct epithelium acts as a barrier to the attainment of an equilibrium

Table 8. Computed estimates of tissue compartment concentrations of urea and sodium chloride[a]

	Measured				Computed for inner portion of inner medulla (IM-3) from models described in text											
					Central core urea *mmoles/ml*			Central core NaCl *μEq/ml*			Loop of Henle NaCl *μEq/ml*		Central core urea / Urine urea			
Condition/rabbit	U_{Osm} *μOsm/g H_2O*	$\frac{U_{Osm}}{P_{Osm}}$	U_{Urea} *mmoles/ml*	IM-3 *mmoles/g H_2O*	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Min.	Max.	Mean
Antidiuresis																
Rabbit 3969	2200	7.3	1169	533	260	488	374	1271	1939	1605	1636	2046	0.22	0.41	0.32
Rabbit 3971	2428	8.28	1094	373	64	317	191	1625	2364	1995	1856	2320	0.06	0.28	0.17
Rabbit 3973	2303	7.78	1115	585	357	550	453	1291	1945	1618	1742	2178	0.32	0.49	0.41
Low-urea diuresis																
Rabbit 3433	1902	6.44	1528	540	116	464	290	1057	1785	1421	1454	1818	0.08	0.30	0.19
Rabbit 3442	1349	4.46	1060	292	0	230	115	848	1386	1117	1000	1250	0	0.21	0.11
Rabbit 3439	1364	4.47	1122	417	114	363	239	727	1249	988	987	1234	0.10	0.32	0.21
Rabbit 3440	1489	4.72	1118	428	132	326	254	815	1356	1086	1073	1342	0.11	0.33	0.22
High-urea diuresis																
Rabbit 3968	653	1.77	430	233	148	220	184	301	504	403	397	496	0.34	0.51	0.43
Rabbit 3970	713	2.07	473	301	227	292	260	278	485	382	447	559	0.48	0.61	0.55
Low-mannitol diuresis																
Rabbit 3394	1657	5.89	282	179	134	173	154	1151	1522	1337	1320	1651	0.47	0.61	0.54
Rabbit 3587	1917	6.58	437	317	265	313	289	1120	1651	1436	1475	1844	0.60	0.71	0.60
Rabbit 3441	1678	6.10	284	224	198	223	211	1112	1479	1296	1366	1708	0.69	0.78	0.74
High-mannitol diuresis																
Rabbit 3580	720	2.44	70.4	43.5	31	42	37	533	688	611	512	641	0.45	0.59	0.52
Rabbit 3581	680	2.20	53.8	20.4	6	17	12	526	673	600	478	598	0.11	0.33	0.22
Rabbit 3582	788	2.59	79	49.6	37	48	43	582	751	667	543	679	0.46	0.60	0.53

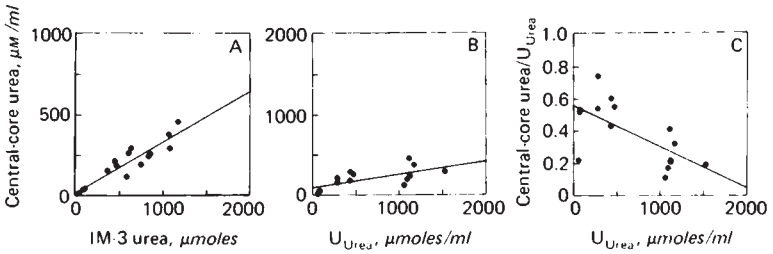

Fig. 7. *Means values of central-core urea and central-core urea/urine urea computed from model related to experimentally measured values of IM-3 urea (papilla) and urine urea.* Regression lines were determined by method of least squares.

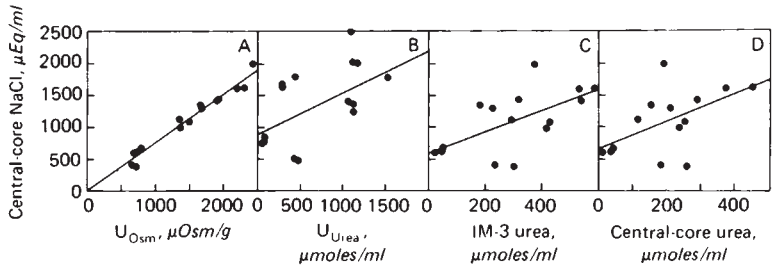

Fig. 8. *Mean values of central core sodium chloride concentration computed from model related to experimentally measured urine osmolality, urine urea concentration, and IM-3 (papilla) urea concentration and to computed central-core urea concentration.*

distribution of urea. At lower urine urea concentrations, this barrier is less effective. Two interpretations appear possible: one, that the diffusional permeability of the collecting duct to urea may decrease as the urea concentration in collecting duct fluid increases and, second, that a carrier-mediated urea transport system in the collecting duct is responsible for urea reabsorption from the inner medullary collecting duct and is saturated at a urine urea concentration of 300 to 400 mmoles. The presence of a carrier-mediated transport system for urea in the mammalian collecting duct has been suggested by several observations [53–55] but not supported by others [56, 57]. The present data do not show any clear relation of the ratio central core urea : urine urea to the urine flow rate or to the urea excretion rate.

A functional barrier to urea transport from collecting duct to central core that exists when the urine urea concentration is elevated can explain the absence of a quantitatively significant enhancement of concentrating ability during urea administration. According to the passive salt accumulation model, there is a high permeability of the inner medullary collecting duct to urea, with a large resultant efflux of urea into the central core. Although Rocha and Kokko found that the collecting duct in the inner medulla had a higher urea permeability than the outer medullary collecting duct did, they emphasized that their permeability results were "only directionally compatible with the previously proposed passive equilibration model and, therefore, this should not be misconstrued as rigorous proof of the proposed model" [11].

The mean central core sodium chloride concentration constitutes a large and constant fraction (approximately 0.76) of the urine osmolality, Fig. 8A, mean central core sodium chloride = 0.76 U_{Osm} − 5.9 (r = 0.98). However, the mean central core sodium chloride concentration is not closely related to either the urine urea concentration (Fig. 8B), the IM-3 urea concentration (Fig. 8C), or the computed central core urea concentration (Fig. 8D): mean central core sodium chloride = 0.58 U_{urea} + 715 (r = 0.56), 1.68 IM-3 urea + 595 (r = 0.63), and 2.16 cc urea + 655 (r = 0.55), respectively. The independence of the computed central core sodium concentration from the urine urea concentration and the central core urea concentration is also contrary to the prediction of the passive salt accumulation model.

Despite findings contrary to major predictions of the passive salt accumulation model, the present results are compatible with either active or passive reabsorption of sodium chloride from the thick ascending limb. Although the entire model calls for a high concentration of urea within the central core, for a large efflux of urea from the collecting duct to

central core, and for a parallelism between the urine urea concentration on the one hand, and the central core sodium chloride and urea concentrations on the other hand, passive salt reabsorption depends solely on the presence of a downhill electrochemical gradient for sodium chloride between thin-ascending-limb fluid and adjacent central core. Conceivably, the urea concentration within the central core could be sufficient, though low, to engender the requisite abstraction of water from the descending limb and elevate the sodium chloride concentration at the tip of the loop to values above the adjacent central core. A comparison of the computed sodium chloride concentrations from the loop of Henle (columns 11 and 12, Table 8) with that from the central core (columns 8 and 9, Table 8) shows that the estimated sodium chloride concentration at the tip of the loop closely approximates, but exceeds, in a majority of comparisons, that from the central core. These computations were based on the assumption that osmotic concentration of the descending limb fluid occurred solely by water abstraction in these rabbits. Therefore, passive sodium chloride reabsorption from the thick ascending limb in these rabbits cannot be ruled out.[1] If osmotic concentration of the descending limb fluid involved, however, significant entry of urea in addition to or in place of water abstraction, then the salt gradient would be reversed, with a higher salt concentration in the central core than in the tip of the loop. Active salt reabsorption would then be required to produce the large accumulation of sodium chloride in the inner medullary central core of these rabbits. A choice of processes is not possible at present because the in vitro results of Kokko [9] are in direct conflict with those of Stoner and Roch-Ramel [49] regarding the potential entry of urea into the rabbit thin descending limb.

Both the carnivorous dog and omnivorous rat may commonly ingest large quantities of meat over short periods when they are in the wild state, whereas the herbivorous sheep and rabbit more commonly graze over lengthy periods of the day. This may be pertinent when considering the large difference between the effect of urea on concentrating ability in the dog and rat, on the one hand, and on sheep and rabbit, on the other. That an evolutionary development of the renal concentrating mechanism has arisen to accommodate differences in the temporal generation of urea would not be surprising, and this possibility deserves further comparative physiologic study.

[1] Dr. J. L. Stephenson called our attention to the possibility that passive salt reabsorption could exist despite a restricted transport of urea from collecting duct to central core.

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Appendix

Description of model

We propose a model of the inner medulla (papilla tip) that will consist of only two fluid compartments. One is the fluid within the collecting duct lumen (designated compartment A, with a urea concentration A_{urea} and a fractional volume F_A). The second compartment is the remaining fluid (designated compartment B, with a urea concentration B_{urea} and a fractional volume F_B). The sum of $F_A + F_B = 1$. The concentration of urea in B is assumed to equal the urea concentration in the central core. The collecting duct concentration of urea is taken to be a function of the measured urinary urea concentration: $A_{urea} = U_{urea} \cdot k_1$. In the following equation, $IM\text{-}3_{urea}$ is the measured concentration of urea in the entire tip of the inner medulla. For the entire papilla

$$IM\text{-}3_{urea} = F_A \cdot A_{urea} + F_B \cdot B_{urea} \qquad (1)$$

and

$$B_{urea} = \frac{IM\text{-}3_{urea}}{F_B} - \frac{F_A \cdot k_1 \cdot U_{urea}}{F_B} \qquad (2)$$

Once obtained, the central core urea concentration can be used to obtain a provisional estimate of the central core sodium concentration. The following approximations are made: *(1)* the only solutes in the central core are sodium chloride and urea; *(2)* the osmotic coefficients of sodium chloride and urea are unity, that is, 2 (Na) + (urea) = (osmolality); and the central core osmolality = $k_2 \cdot$ urine osmolality. Equation 3 follows:

$$\text{central core}_{NaCl} = (k_2 \cdot \text{urine osmolality}) - B_{urea} \qquad (3)$$

The concentration of sodium chloride at the tip of the loop of Henle in the inner medulla will depend on the process by which the loop fluid is made hyperosmotic. The highest sodium chloride concentrations at the bend of the loop will be obtained if the entire descending limb is impermeable to sodium chloride and urea but permeable to water, thus bringing about osmotic equilibration only by the movement of water. This process is required by the passive salt accumulation hypotheses. If it is assumed that this process occurs in the rabbits in group 4, it is possible to compute this upper limit to the loop sodium chloride concentration in IM-3, recognizing *(1)* that at the beginning of the loop the tubular fluid sodium chloride concentration can be equated to $2 \cdot P_{Na}$, *(2)* that at the tip of the loop the sodium concentration has increased in proportion to the increase in tubular fluid osmolality, that is, $\text{loop tip}_{NaCl}/\text{plasma}_{NaCl} = IM\text{-}3_{Osm}/P_{Osm}$, and *(3)* $IM\text{-}3_{Osm} = k_2 \cdot U_{Osm}$. Equation 4 follows:

$$\text{loop tip}_{NaCl} = 2 \cdot P_{Na} \cdot \frac{U_{Osm}}{P_{Osm}} \cdot k_2 \qquad (4)$$

Evaluation of model

To evaluate equation 2, values for the parameters F_A and k_1 must be chosen. Measurements of the fractional volumes of the different anatomic compartments of the rabbit papilla during normal function have not been made. Knepper et al [51], however, have measured volumes in rabbit kidneys removed and fixed after induction of a high mannitol diuresis and clamping of the renal vein. Pfaller and Rittinger [52] have reported fractional volumes in excised and fixed rat kidneys. Collecting duct luminal volumes of 8 to 12% [51] and of 3 to 6% [52] of the total papilla tip tissue volume were found. In both studies the volume of the collecting duct lumen is likely to be underestimated due to run-out of fluid from the ducts after excision of the kidneys. Consequently, we have assumed for our computations that the fractional volume of the collecting ducts (F_A) was likely to be within the limits 0.1 to 0.3. The precise relation of solute concentration in the terminal collecting duct to that in the urine is unknown in our rabbits, but from the observed cortex-to-papilla urea gradients, it appears that the mean collecting duct urea concentration in IM-3 may lie between 0.8 and 1.0 times the urinary urea concentration. Therefore, we have used 0.8 and 1.0 for minimum and maximum values of k_1 in our model. Having made these assumptions regarding minima and maxima for F_A and k_1, and using the measured values for $IM\text{-}3_{urea}$ and U_{urea}, we computed maxima and minima for the central core urea concentration (B_{urea}) for each rabbit. These values are listed in Table 8, columns 5 and 6. When negative values for central core urea concentration were obtained, a minima of zero was listed. As a measure of central tendency, the mean of the maxima and minima were also computed (column 7).

The accuracy of the estimates of the central core urea concentration based on this model is open to obvious criticism. There are more than two dissimilar fluid compartments in the inner medula, and neither the fractional volume values or values of k_1 that were used were experimentally determined. From studies on other mammals fed high or normal protein diets, however, it appears the urea concentration diminishes downwards through the following anatomical units: collecting duct lumen to collecting duct cell to interstitial fluid and vasa recta blood to interstitial cell and loop of Henle epithelium and, lastly, to loop of Henle tubular fluid [16, 17]. If it is assumed that this gradient exists in our rabbits, then it seems reasonable to expect the interstitial cell urea concentration will closely approximate the central core urea concentration, and the central core urea concentration will exceed the urea concentration in the loop of Henle and will be less than the collecting duct cell urea concentration. The central tendency of the urea concentration in loop fluid and collecting duct cell would be some intermediate value, a value closer to the central core urea concentration than either extreme. These considerations support the approximation of an equality of urea concentration between central core and the entire compartment B.

To compute *maximum* values of the central core sodium chloride concentration (Equation 3), we used the minimum value of B_{urea} and $k_2 = 1.0$. To compute the *minimum* values of central core sodium chloride concentration, we used the maximum value of B_{urea} and $k_2 = 0.8$. The resulting computed values are given in columns 8 and 9 of Table 8; column 10 gives the mean central core sodium chloride concentration.

Additional support for the values chosen for k_1 and F_A arises from the recognition that the measured concentration of any solute within the papilla can be expressed in terms of N compartments, each with its own concentration of solute, and that the concentration in the collecting duct fluid (designated compartment A) can be expressed as a function of the measured urinary concentration. Thus, for urea,

$$IM\text{-}3_{urea} = F_A \cdot k_1 \cdot U_{urea} + (F_{B_1} \cdot B_{1_{urea}} + F_{B_2} \cdot B_{2_{urea}} + F_{B_N} \cdot B_{N_{urea}}). \quad (5)$$

A regression plot of IM-3 against U for any substance, when analyzed by the method of least squares for a straight line relationship, will give a slope of $F_A \cdot k_1$. When this relationship was determined for the antidiuretic and low-diuresis experiments, the slopes and correlation coefficients obtained were: urea, 0.27 (r = 0.82, N = 10); sodium, 0.24 (r = 0.60, N = 11); potassium, 0.16 (r = 0.49, N = 11). The slopes ($F_A \cdot k_1$) for all three substances were similar (range, 0.16 to 0.27). In the computations for the central core urea concentrations, $F_A \cdot k_1$ was varied from a maximum of $0.3 \cdot 1 = 0.3$, to a minimum of $0.1 \cdot 0.8 = 0.08$, and thus the maximum and minimum bracketed and closely approximated the values of ($F_A \cdot k_1$) derived from our experimental observations.

To evaluate Equation 4, we used 0.8 and 1.0 for the limits of k_2. This provided the minimum and maximum estimates of tip loop sodium chloride concentration listed in Table 8, columns 11 and 12.

Table 8 presents estimates of central core urea and sodium obtained from application of this model. Despite their provisional nature, the computed estimate of the central core urea and sodiums concentrations will be closer to the true central core urea concentration than the measured urea concentration in IM-3 (the papilla tip) will be. It is therefore a more useful quantity than the total papilla tip concentration commonly used in previous analyses of medullary urea distribution.

In summary, the estimates of central core urea and sodium concentrations obtained from the application of the model (Table 8) are provisional but are likely to be closer to the true central core urea and sodium concentrations than the measured papillary concentrations previously used in studies of medullary function [20, 29, 30, 44, 45, 55] will be.

References

1. ANDREOLLI TE, BERLINER RW, KOKKO JP, MARSH DJ: Questions and replies: renal mechanisms for urinary concentrating and diluting processes. *Am J Physiol* 235:F1-F11, 1978
2. BERLINER RW: The concentrating mechanism in the renal medulla. *Kidney Int* 9:214-222, 1976
3. JAMISON RL: Countercurrent systems, in *MIP International Review of Science*, edited by THURAU K, Baltimore, University Park Press, 1974, series 1, vol. 6 (Kidney and Urinary Tract Physiology), pp. 199-245
4. RECTOR FC JR: Renal concentrating mechanisms, in *Disturbances in Body Fluid Osmolality*, edited by ANDREOLLI TE, GRANTHAM JJ, RECTOR FC JR, Bethesda, *Am Physiol Soc*, 1977, pp. 179-196
5. BERLINER RW, BENNETT CM: Concentration of urine in the mammalian kidney. *Am J Med* 42:777-789, 1967
6. STEPHENSON JL: Concentration of urine in a central core model for the renal counterflow system. *Kidney Int* 2:85-94, 1972
7. STEPHENSON JL: Countercurrent transport in the kidney. *Ann Rev Biophys Bioeng* 7:315-339, 1978
8. KOKKO JP, RECTOR FC JR: Countercurrent multiplication system without active transport in inner medulla. *Kidney Int* 2:214-223, 1972
9. KOKKO JP: Sodium chloride and water transport in the descending limb of Henle. *J Clin Invest* 49:1838-1846, 1970
10. IMAI M, KOKKO JP: NaCl, urea and H_2O transport in the thin ascending limb of Henle: generation of osmotic gradients by passive diffusion of solutes. *J Clin Invest* 53:393-402, 1974
11. ROCHA AS, KOKKO JP: Permeability of medullary nephron segments to urea and water: Effect of vasopressin. *Kidney Int* 6:379-387, 1974
12. MARSH DJ: Solute and water flows in thin limbs of Henle's loop in the hamster kidney. *Am J Physiol* 218:824-831, 1970
13. PENNELL JP, LACY FB, JAMISON RL: An in vivo study of

the concentrating process in the descending limb of Henle's loop. *Kidney Int* 5:337-347, 1974
14. JAMISON RL, BUERKERT J, LACY F: A micropuncture study of Henle's loop in Brattleboro rats. *Am J Physiol* 224:180-185, 1973
15. DEROUFFIGNAC C: Physiological role of the loop of Henle in urinary concentration. *Kidney Int* 2:297-303, 1972
16. DEROUFFIGNAC C, MOREL F: Micropuncture study of water, electrolytes, and urea movements along the loops of Henle in Psammomys. *J Clin Invest* 48:474-486, 1969
17. GOTTSCHALK CW, LASSITER WE, MYLLE M, ULLRICH KJ, SCHMIDT-NIELSEN B, O'DELL R, PEHLING G: Micropuncture study of composition of loop of Henle fluid in desert rodents. *Am J Physiol* 204:532-535, 1963
18. LASSITER WE, MYLLE M, GOTTSCHALK CW: Micropuncture study of urea transport in rat renal medulla. *Am J Physiol* 210:965-970, 1966
19. STEWART J, VALTIN H: Computer simulation of osmotic gradient without active transport in renal inner medulla. *Kidney Int* 2:264-270, 1972
20. BRAY GA, PRESTON AS: Effect of urea on urine concentration in the rat. *J Clin Invest* 40:1952-1960, 1961
21. CRAWFORD JD, DOYLE AP, PROBST JH: Service of urea in renal water conservation. *Am J Physiol* 196:545-548, 1959
22. EDELMAN CM JR, BARNETT HL, START H: Effect of urea on concentraton of urinary nonurea solute in premature infants. *J Appl Physiol* 21:1021-1025, 1966
23. EDELMAN CM JR, BARNETT HL, TROUPKOU V: Renal concentrating mechanisms in newborn infants. Effect of dietary protein and water content, role of urea, and responsiveness to antidiuretic hormone. *J Clin Invest* 39:1062-1069, 1960
24. EPSTEIN FH, KLEEMAN CR, PURSEL S, HENDRIKX A: The effect of feeding protein and urea on the renal concentrating process. *J Clin Invest* 36:635-641, 1957
25. GAMBLE JL, MCKHANN CF, BUTLER AM, TUTHILL E: An economy of water in renal function referable to urea. *Am J Physiol* 109:139-154, 1934
26. HENDRIKX A, EPSTEIN FH: Effect of feeding protein and urea on renal concentrating ability in the rat. *Am J Physiol* 195:539-542, 1958
27. JAENIKE JR: Urea enhancement of water reabsorption in the renal medulla. *Am J Physiol* 199:1205-1210, 1960
28. KOIKE TI, KELLOGG RH: Effect of urea loading on urine osmolality during osmotic diuresis in hydropenic rats. *Am J Physiol* 205:1053-1057, 1963
29. LEVINSKY NG, BERLINER RW: The role of urea in the urine concentrating mechanism. *J Clin Invest* 38:730-740, 1959
30. RABINOWITZ L: Enhancement of renal concentrating ability in the rat by acetamide and methylurea. *Am J Physiol* 214:737-744, 1968
31. RABINOWITZ L, KELLOGG RH: Enhancement of renal concentrating ability in the dog by urea and related compounds. *Am J Physiol* 205:112-116, 1963
32. REINHARDT HW, KLOSE HJ, ELLINGHAUS K, SCHAD H, ARMSEN T: Die Konzentrierung des Harnstoffes in der Niere I: Clearanceuntersuchungen an wachen Hunden. *Pfluegers Arch* 310:320-330, 1969
33. STEINMETZ PR, SMITH HW: Urea and the renal concentrating operation in man. *Am J Med* 35:727-736, 1963
34. RABINOWITZ L, GUNTHER RA: Renal concentrating ability in sheep during urea, mannitol, and methylurea diuresis. *Am J Physiol* 222:801-806, 1972
35. MCKINLEY JE, GILBERT DB, CHAO PY, REEVE EB: Urea metabolism and distribution in rabbits treated with neomycin. *Am J Physiol* 218:491-497, 1970
36. RABINOWITZ L, GUNTHER RA, SHOJI ES, FREEDLAND RA, AVERY EH: Effects of high and low protein diets on sheep renal function and metabolism. *Kidney Int* 4:188-207, 1973
37. BARRACLOUGH MA, GUIGNARD J-P, JONES NF: Urine concentration during solute diuresis in potassium-depleted rabbits: Evidence for a defect in tubular sodium transport. *J Physiol (Lond)* 212:763-775, 1971
38. AIZAWA C, HONDA N, YOSHITOSHI Y: Depletion of the renal medullar osmotic gradient following hemorrhagic hypotension in hydropenic rabbits. *Japan Heart J* 10:177-184, 1969
39. FORREST JN, STANIER MW: Kidney composition and renal concentrating ability in young rabbits. *J Physiol (Lond)* 187:1-4, 1966
40. HEALY JK, ELLIOTT AJ: The effect of a natriuretic dose of angiotensin on rabbit kidney composition. *Clin Sci* 38:727-739, 1970
41. NIHEI H, HONDA N, SUZUKI K, NAGASE M, YOSHITOSHI Y: Renal hemodynamics and medullary osmotic gradient in ischemic acute renal failure in rabbits. *Japan Heart J* 16:44-56, 1975
42. NOCENTI MR, CIZEK LJ: Electrolyte-fluid exchange and renal tissue composition in vasopressin polyuric-polydipsic rabbits. *Proc Soc Exp Biol Med* 124:767-770, 1967
43. SAKAI F, ENDOU H: Postnatal development of urea concentration in the newborn rabbit's kidney. *Japan J Pharmacol* 21:677-679, 1971
44. SCHMIDT-NIELSEN B, O'DELL R: Structure and concentrating mechanism in the mammalian kidney. *Am J Physiol* 200:1119-1124, 1961
45. SCHMIDT-NIELSEN B, O'DELL R, OSAKI H: Interdependence of urea and electrolytes in production of a concentrated urine. *Am J Physiol* 200:1125-1132, 1961
46. URAKABE S, ORITA Y, FURUKAWA T, ABE H: Analysis of urea excretion with special reference to the influence of abrupt change in urine flow. *Japan Circ J* 29:923-930, 1965
47. SMITH HW: *The Kidney: Structure and Function in Health and Disease*. New York, Oxford, 1951, pp. 535-538
48. FOSTER DM, JACQUEZ JA: Comparison using central core model of renal medulla of the rabbit and rat. *Am J Physiol* 234:F402-F414, 1978
49. STONER LC, ROCH-RAMEL F: Properties of rabbit descending limb of Henle. Abstract. *Kidney Int* 14:782, 1978
50. ROCH-RAMEL F, FILLOUX B, GUIGNARD JP, PETERS G: Fate of urea in Henle's loops of the rabbit and the rat, in *New Aspects of renal function*, edited by VOGEL HG, ULLRICH KJ, Amsterdam-Oxford, Excerpta Medica, 1978, p. 118
51. KNEPPER MA, DANIELSON RA, SAIDEL GM, POST RS: Quantitative analysis of renal medullary anatomy in rats and rabbits. *Kidney Int* 12:313-323, 1977
52. PFALLER W, RITTINGER M: Quantitative Morphologie der Niere. *Mikroskopie (Wien)* 33:74-79, 1977
53. SCHMIDT-NIELSEN B, OSAKI H, MURDAUGH HV JR, O'DELL R: Renal regulation of urea excretion in sheep. *Am J Physiol* 194:221-228, 1958
54. ULLRICH KJ, RUMRICH G, SCHMIDT-NIELSEN B: Urea transport in the collecting duct of rats on normal and low protein diets. *Pfluegers Arch* 293:147-156, 1967
55. GOLDBERG M, WOJTCZAK AM, RAMIREZ MA: Uphill transport of urea in dog kidney: Effect of certain inhibitors. *J Clin Invest* 46:388-399, 1967
56. RABINOWITZ L: Mechanism of renal excretion of methylurea and acetamide in the dog. *Am J Physiol* 209:188-194, 1965
57. RABINOWITZ L, GUNTHER RA: Urea and sodium in sheep kidneys during ethacrynic acid diuresis. *Pfluegers Arch* 353:349-360, 1975